Proteome Analysis of Skin Distinguishes Acute Guttate from Chronic Plaque Psoriasis

Lina M. Carlén,* Fabio Sánchez,* Ann-Charlotte Bergman,† Susanne Becker,‡ Daniel Hirschberg,§ Bo Franzén,¶ Jonathan Coffey,# Hans Jörnvall,§ Gert Auer,‡ Ayodele A. Alaiya,*‡ and Mona Ståhle*

Departments of *Dermatology and †Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden; ‡Unit of Cancer Proteomics, Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden; §Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden; ¶Molecular Sciences, AstraZeneca, Södertälje, Sweden; #Waters Ltd, Manchester, UK

Psoriasis is a disease with considerable heterogeneity in clinical presentation. This is the first study using two-dimensional gel electrophoresis to compare global protein expression patterns in lesional and non-lesional skin from subjects with acute guttate psoriasis associated with streptococcal throat infection and chronic plaque psoriasis. Samples from experimentally induced contact eczema and normal skin from healthy controls were also included. Proteins with statistically significant differences in expression were used in hierarchical cluster analyses resulting in separation of the different samples into groups. Chronic plaque and guttate psoriasis samples were distinctly separated, indicating that they represent discrete phenotypes at the protein expression level. Interestingly, there was a trend in which guttate psoriasis lesions clustered closer to eczema than to chronic plaque psoriasis lesions, indicating that the duration of the inflammatory reaction may affect clustering. Several of the differentially expressed proteins were identified by mass spectrometry.

Key words: autoimmunity/cluster analysis/2D PAGE/inflammation/skin disease
J Invest Dermatol 124:63–69, 2005

Psoriasis is a common skin disorder that affects 2%–3% of western population. There is substantial clinical variation and heterogeneity in disease presentation. The most common phenotype is chronic plaque psoriasis with well-demarcated lesions typically located in the scalp and on the extensor surfaces. Another disease manifestation is guttate psoriasis that presents itself as sudden dissemination of small, red, and scaly papules. Guttate psoriasis is frequently identified as the first manifestation of the disease in younger individuals and is reported to develop into chronic plaque psoriasis in a large proportion of patients (Williams *et al*, 1976). Flares of guttate psoriasis can also occur in individuals with chronic plaque psoriasis. The etiology and pathogenesis of psoriasis are still unclear. The inflammatory process in skin is characterized by an influx of T cells and neutrophils and a switch of the keratinocyte phenotype towards dedifferentiation and hyperproliferation. Previously regarded as an epidermal disorder, psoriasis is now primarily considered to be an autoimmune reaction, of which the precise mechanism(s) remain to be solved (Valdimarsson *et al*, 1995; Prinz, 1999).

The genetic contribution to psoriasis is high, and several psoriasis susceptibility loci (PSORS1–9) on different chromosomes have been identified in genome-wide linkage studies (HUGO Gene Nomenclature Committee; http://www.gene.ucl.ac.uk/nomenclature). A major susceptibility locus is the PSORS1 on chromosome 6p21.3 (Nair *et al*, 1997; Trembath *et al*, 1997). This locus contains the human leukocyte antigen (HLA) genes, and a specific allele of *HLA-C* (*Cw*0602*) (Trembath *et al*, 1997; Bhalerao and Bowcock, 1998) has been shown to be highly associated with psoriasis. The genetic background in psoriasis appears to be complex and whether different psoriasis phenotypes are associated with distinct genotypes is still unclear. In addition to genetic susceptibility, environmental factors such as infection (Gudjonsson *et al*, 2003), skin trauma (Eyre and Krueger, 1982), and stress (Seville, 1989; Naldi *et al*, 2001) are thought to be responsible for development of the disease. Streptococcal throat infections are particularly associated with the manifestation of guttate psoriasis (Whyte and Baughman, 1964; Telfer *et al*, 1992) but are also reported to exacerbate and maintain chronic plaque psoriasis (Gudjonsson *et al*, 2003).

Global gene expression in psoriasis has been investigated in several studies. When comparing lesional and non-lesional skin from patients with chronic plaque psoriasis and skin from healthy controls, differentially regulated transcripts were identified (Bowcock *et al*, 2001; Oestreicher *et al*, 2001; Zhou *et al*, 2003). Based on differences in gene expression levels, involved skin from psoriasis patients and healthy skin from controls were differentiated by hierarchical cluster analysis (Bowcock *et al*, 2001; Oestreicher *et al*, 2001). Recently, gene expression profiles were reported to differ between skin lesions from patients with atopic

Abbreviations: Ambic, ammonium bicarbonate; ACN, acetonitrile; 2DE, two-dimensional gel electrophoresis; GST-π, glutathione S-transferase P; HSP, heat shock protein; RhoGDI, RhoGDP dissociation inhibitor; SCCA-2, squamous cell carcinoma antigen-2; WW, wet weight

dermatitis and psoriasis (Nomura *et al*, 2003). At the protein level, there have been attempts to characterize expression patterns in psoriasis. Psoriatic keratinocytes have been investigated by two-dimensional gel electrophoresis (2DE) (Celis *et al*, 1990a, b) and in other studies, proteins with differential expression patterns between lesional psoriatic and normal skin have been identified (Madsen *et al*, 1991; Madsen *et al*, 1992). There have been no studies, however, comparing the global protein expression patterns in patients with well-defined and characterized psoriasis phenotypes in relation to experimentally induced skin inflammation.

In this study, the 2DE technique was used to analyze global protein expression patterns in skin biopsies from individuals with distinct psoriasis phenotypes, acute contact eczema and healthy controls. Proteins with statistically significant differences in expression patterns were used in hierarchical cluster analysis in order to separate the groups. Several of the differentially expressed proteins were identified by mass spectrometry.

Results

Four differentially expressed protein sets were found after performing the following comparisons through Mann–Whitney analysis and Student's *t* test; lesional plaque (n = 7) *versus* lesional guttate (n = 6) protein profiles (60 proteins), lesional guttate (n = 6) *versus* lesional eczema (n = 4) protein profiles (64 proteins), lesional guttate (n = 6) *versus* non-lesional guttate (n = 5) protein profiles (34 proteins) and lesional plaque psoriasis (n = 7) *versus* lesional eczema (n = 4) protein profiles (164 proteins).

Identified proteins Twenty-one proteins were identified in this study (Table S1), of which 12 were found to have more than 2-fold average up- or downregulation in any group compared with normal skin (Table I). The selection was based on the densitometric quantification of the protein spots. All the identified proteins are marked in the 2D gel image in Fig 1.

The identified proteins in the 60 protein sets include calreticulin, cytokeratins 10 and 15, two variants of squamous cell carcinoma antigen-2 (SCCA-2), also known as leupin, glutathione S-transferase P (GST-π), maspin, RhoGDP dissociation inhibitor (RhoGDI), and heat shock protein-27 (HSP27). The two SCCA-2 variants differ in pI by approximately 0.2 units, have similar molecular weights and were upregulated in plaque, guttate, and eczema lesions compared with normal skin. The higher pI variant showed a 38- and 171-fold upregulation in lesional guttate and plaque, respectively. The lower pI variant showed a 31- and 139-fold upregulation, respectively. Calreticulin was downregulated in lesional plaque and guttate psoriasis and in non-lesional guttate psoriasis skin compared with normal skin, but slightly upregulated in eczema (Table I). RhoGDI was upregulated in plaque and guttate psoriasis and also in eczema lesions. Cytokeratins 10 and 15 were downregulated in plaque and guttate lesions compared with normal, whereas GST-π, maspin and HSP27 were slightly upregulated in lesional plaque psoriasis.

Table I. Proteins with more than 2-fold change in plaque psoriasis compared with normal skin

Protein	Lesional plaque	Lesional guttate	Non-lesional guttate	Eczema
SCCA-2, high pI	**171**	**38**	**1.8**	**4.3**
SCCA-2, low pI	**139**	**31**	−5.5	**3.4**
Maspin	**1.9**	−1.3	−1.1	−2.1
Cytokeratin 10	ND	−9	−2.8	−1.6
Cytokeratin 14	**30**	**4**	−1.1	−1.6
Cytokeratin 15	−15	−1.3	**1.2**	−1.3
Cytokeratin 17	**9**	**5**	−1.5	**1.4**
GST-π	**2**	−1.1	−1.2	−1.3
HSP27	**2**	−1.1	**1.2**	−1.5
RhoGDI 1	**11**	**5**	**2.3**	**12.4**
Calreticulin	−3	−1.1	−1.9	**1.9**
14–3–3 σ	**3**	−1.4	−1.4	**1.1**

The fold change between groups is calculated on the basis of average expression level in each sample type.
SCCA-2, squamous cell carcinoma antigen-2; GST, glutathione S transferase; HSP, heat shock protein; RhoGDI, RhoGDP dissociation inhibitor; ND, not detected; bold, upregulated; –, downregulated.

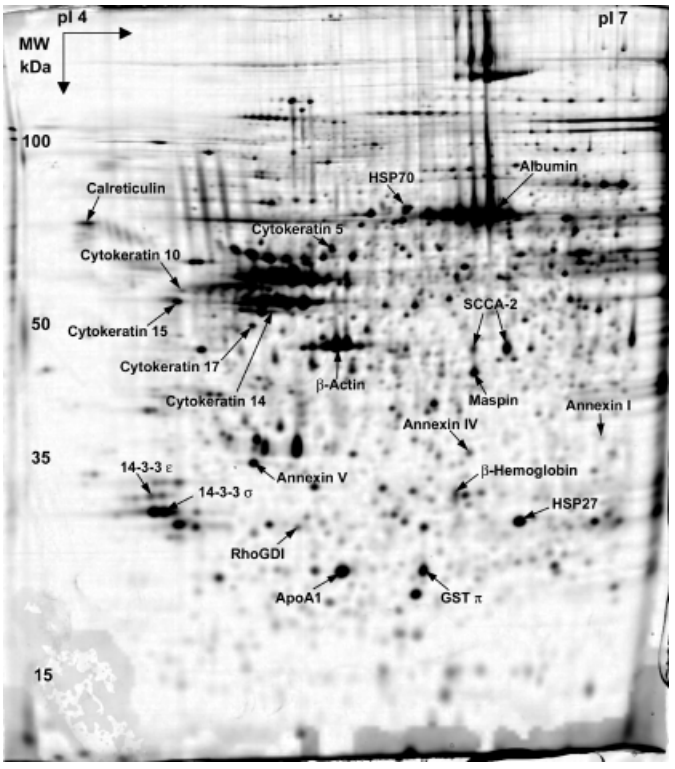

Figure 1
Representative silver-stained two-dimensional (2D) gel image from lesional guttate psoriasis skin showing the position of the identified proteins. pI (isoelectric point), kDa (kilo-Dalton), SCCA-2 (squamous cell carcinoma antigen-2), GST (glutathione S transferase), HSP (heat shock protein), RhoGDI (RhoGDP dissociation inhibitor), Apo A1 (Apolipoprotein A1).

Calreticulin and the two variants of SCCA-2 were identified in the set of 64 proteins, whereas in the 34-protein set two variants of SCCA-2 and cytokeratin 17 were identified.

Cytokeratin 17 was upregulated in lesional skin from plaque and guttate psoriasis patients compared with normal skin from controls (Table I).

Based on these protein data sets, hierarchical cluster analysis was performed including profiles from all lesional and non-lesional samples and normal tissue (Table S2). All four comparisons presented above generated protein data sets that produced highly similar dendrograms, indicating that such protein sets were not likely detected by chance (Fig 2).

Lesional skin from plaque and guttate psoriasis and induced contact eczema separate by proteome analysis All cluster analyses resulted in a clear separation of lesional plaque psoriasis samples from all other groups. In addition, profiles from lesional guttate and lesional eczema clustered in the same branch, although as distinguishable phenotypes (Fig 2). The fact that samples within each group clustered together indicates that the protein data sets used are biologically relevant.

Lesional plaque psoriasis is clearly distinct from all other groups All comparisons showed that plaque psoriasis lesions consistently agglomerated under one separate branch, whereas the other phenotypes were grouped on a different cluster (Fig 2). In contrast, non-lesional samples from the different groups did not show a consistent clustering pattern but were clearly different from plaque lesions.

Lesional skin from guttate psoriasis clusters closer to contact eczema than to plaque psoriasis All dendrograms show that lesional guttate and lesional eczema samples clustered under the same branch and were consistently positioned separate from plaque psoriasis. Uninvolved and normal samples, however, appeared to distribute in an inconsistent manner slightly affecting the grouping around lesional eczema and lesional guttate samples (Fig 2). This may be due to the small sample size decreasing the discriminative power of the analysis.

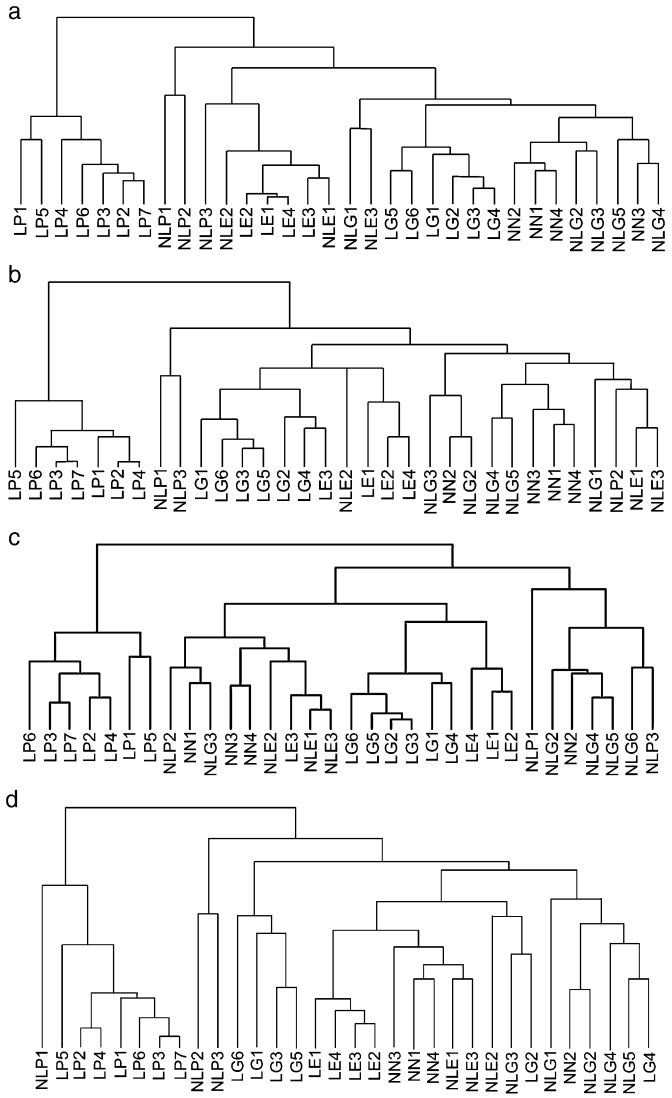

Figure 2
Skin protein profiles from plaque psoriasis, guttate psoriasis and contact eczema lesions separate by hierarchical cluster analysis. All dendrograms show that plaque lesions are distinct from the other phenotypes and that guttate psoriasis and contact eczema are positioned under the same branch. (*a*) The cluster analysis was based on 64 proteins with significantly different expression level between lesional skin from patients with guttate psoriasis and contact eczema. (*b*) The cluster analysis was based on 60 proteins with significantly different expression level between lesional guttate and lesional plaque. (*c*) Cluster analysis based on 34 proteins differentially expressed between lesional and non-lesional guttate psoriasis. (*d*) Cluster analysis based on 164 proteins with significantly different expression level between lesional plaque and lesional eczema. NN (normal), NLG (non-lesional guttate), LG (guttate), NLE (non-lesional eczema), LE (eczema), NLP (non-lesional plaque), LP (plaque).

Discussion

This study represents the first global protein analysis of skin from patients with distinct psoriasis phenotypes. Current research suggests that there is both clinical and genetic evidence for heterogeneity in psoriasis, but whether psoriasis actually consists of different diseases with distinct etiologic backgrounds has yet to be resolved. There is considerable overlap in phenotype expression and a single individual can display different psoriasis manifestations over time. In this investigation, we chose to study acute guttate psoriasis and stable chronic plaque psoriasis as clearly recognizable clinical phenotypes. We hypothesized that the global protein profiles from such phenotypes would be distinct, reflecting different disease processes. Considerable effort was taken to maximize homogeneity among samples, including only individuals with clear and simple phenotypes and obtaining all skin biopsies from the same body region. Moreover, in all guttate patients, we isolated *Streptococcus pyogenes* in throat cultures as the likely triggering factor. Acute guttate flares can occur in patients with chronic plaque psoriasis and the phenotypes can co-exist in the same individual. Thus, although these phenotypes may represent manifestations of the same disease, our results indicate that they are sufficiently distinct to produce separable signatures of global protein expression. This is supported by the finding that the different comparisons consistently showed similar clustering patters. In addition to the clear separation between plaque and guttate psoriasis lesions, we also found a trend in which guttate psoriasis

samples clustered closer to those of contact eczema than to those of plaque psoriasis. Thus, the duration of the inflammatory reaction in skin, acute *versus* chronic, may affect clustering patterns. Since contact eczema represents a prototypic T helper 1-driven inflammatory reaction (Grewe *et al*, 1998; Cavani *et al*, 2000), its global protein network may show similarities to that of acute psoriasis. This observation underscores the dynamic nature of skin inflammation and is in line with reports from atopic dermatitis, where the inflammatory and cytokine networks undergo significant changes over time (Thepen *et al*, 1996; Grewe *et al*, 1998).

Uninvolved skin from any of the phenotypes did not cluster in a consistent pattern; however, they were all distinct from lesional plaque samples (Fig 2). Also, there was no evidence that healthy skin separated from uninvolved psoriasis or eczema skin, as has been discussed (Uyemura *et al*, 1993; de Boer *et al*, 1994). This may reflect, however, the small samples sizes in this study that may undermine the clustering power. Substantially increasing the number of patients may enhance discrimination.

Some of the proteins identified in this study have been described previously in psoriasis, for example, cytokeratins 10 and 17 as well as SCCA-2. The protein with the largest fold upregulation was SCCA-2. It was highly upregulated in skin from both guttate and plaque psoriasis patients compared with normal skin, which is consistent with recently published microarray analyses on plaque psoriasis (Bowcock *et al*, 2001; Oestreicher *et al*, 2001). SCCA-2 belongs to the serine protease inhibitor superfamily and can be detected in the sera of patients with psoriasis (Hamanaka *et al*, 1997). It inhibits chymotrypsin-like serine proteinases like cathepsin G and mast cell chymase (Schick *et al*, 1997), and may have a protective role against various proteinases in pathophysiological events in psoriasis (Takeda *et al*, 2002). Previous studies have shown that high serum levels of SCCA-2 and the intensity of protein expression in lesional psoriasis skin, correlate with disease activity (Takeda *et al*, 2002).

HSP27 was upregulated in skin from plaque psoriasis patients compared with normal skin from controls. HSP27 is constitutively expressed in epidermis and acts as a chaperone in protein folding and as a regulatory protein in actin polymerization (Jonak *et al*, 2002; Morris, 2002). Psoriatic skin contains various HSP, including HSP27 and 70 (Curry *et al*, 2003), which have recently come into focus for their immunomodulatory functions with potential roles in autoimmune diseases (Asea *et al*, 2000). HSP27 has been shown to induce nuclear factor-κB activity in fibroblasts, which promotes cytokine production leading to T lymphocyte recruitment into the skin (Bell *et al*, 2003).

RhoGDI was upregulated in both guttate and plaque psoriasis skin. This protein was described in keratinocytes by proteome studies (Leffers *et al*, 1993b) and in psoriasis by microarray analysis (Bowcock *et al*, 2001). RhoGDI has recently been implicated in the signalling cascade controlling matrix metalloproteinase-9 in response to keratinocyte injury and wound healing (Turchi *et al*, 2003). The Rho family is involved in cell transformation, motility and adhesion by regulating the actin cytoskeleton. Regulation of the Rho family is controlled by a number of activators and inhibitors. The inhibitors include RhoGDI, which regulates the GDP/GTP exchange reaction.

14–3–3σ was upregulated in lesional plaque psoriasis. The 14–3–3 family regulates protein kinases and other proteins involved in signal transduction pathways. 14–3–3σ has been found in epithelial cells and mainly in stratified squamous keratinizing epithelium (Leffers *et al*, 1993a). Following DNA-damage, 14–3–3σ is upregulated by a p53-dependent mechanism and prevents the cell from entering mitosis (Fu *et al*, 2000).

Calreticulin was downregulated in lesional plaque psoriasis skin. It is a calcium binding protein acting as a chaperone promoting folding of glycoproteins in the endoplasmic reticulum. In addition, it is implicated in calcium storage and signalling. Downregulation of calreticulin has been shown to reduce cell attachment through integrin receptors to extracellular matrix *in vitro* (Leung-Hagesteijn *et al*, 1994).

The expression patterns of cytokeratins in the different samples were as expected. Keratins 14 and 17 were highly upregulated, whereas keratins 10 and 15 were markedly downregulated in plaque psoriasis compared with normal skin.

We have not found discrepancies with other reports in terms of the identity of the gene products detected so far in our study. Concerning the number of identified proteins, discrepancies with microarray studies may be accounted for by technical differences. Although microarray analysis is designed to detect differences in expression patterns within a set of known transcripts, proteomics deals with the analysis of 2D profiles within sets of unknown proteins. We have identified a limited amount of differentially expressed proteins, which are consistent with those reported in the literature. Qualitative discrepancies between proteome and microarray data likely arise from post-transcriptional and post-translational regulation of gene expression (Anderson and Seilhamer, 1997). A number of RNA transcripts and proteins previously described as up- or downregulated in psoriasis skin were not identified in this study, e.g., psoriasin (Madsen *et al*, 1991; Bowcock *et al*, 2001), SCCA-1 (Rivas *et al*, 1997) and psoriasis-associated fatty acid binding protein-5 (Madsen *et al*, 1992). It is possible, however, that these proteins were among the differentially expressed proteins not yet identified in our different data sets. The analysis by 2DE is limited by the fact that the displayed proteins represent a fraction of the actual protein content of the tissue analyzed. In this case, a wide range in protein expression levels as well as differences in solubility limits the number of detected proteins. The charge and size ranges were limited to proteins with a pI between 4 and 7, and a molecular weight of 10–200 kDa. Detection of proteins of low abundance, such as receptors and proteins in signal transduction or regulatory pathways, as well as integral membrane proteins, is limited in this analysis. Despite these limitations, we were able to separate two distinct psoriasis phenotypes by hierarchical cluster analysis and identify proteins with differential expression patterns between the groups. Additional studies are needed to integrate the proteins into functional networks and also to follow dynamics of protein expression through disease stages and development.

Materials and Methods

Tissue samples Details regarding patients and controls are presented in Table S2. Paired biopsies from lesional (n = 6) and non-lesional (n = 5) skin were taken from patients with acute untreated guttate psoriasis. All patients had a concomitant throat infection and *Streptococcus pyogenes* was isolated from the throat in all six patients. Non-lesional skin was obtained at least 10 cm away from the psoriasis lesion. Lesional (n = 7) and non-lesional samples (n = 3) from patients with stable chronic plaque psoriasis, without topical or systemic treatment for at least 2 mo, were also included. Paired samples were obtained from one patient. The plaque psoriasis skin lesions were clinically comparable in thickness with the guttate psoriasis lesions. Control samples were taken from four healthy individuals with no family history of psoriasis as reference for the normal skin protein pattern. In four individuals with known contact allergy to nickel but otherwise healthy, paired biopsy specimens were taken from positive patch test reactions to 5% nickel sulfate, and from non-lesional skin (n = 3). The patch testing was performed in a standardized manner using polypropylene-coated Finn chambers (Epitest, Helsinki, Finland) mounted on Scanpor (Norgeplaster, Oslo, Norway) for 72 h. All skin samples from patients and controls were obtained from the lower back using a 6 mm biopsy punch to a depth of 7 mm and were snap-frozen and stored at -70°C. All biopsies were taken with written informed consent and with approval of the Karolinska Institute Committee of Ethics. The study was conducted according to the Declaration of Helsinki Principles.

Sample preparation The skin biopsies were kept frozen in liquid nitrogen and mechanically homogenized using a mortar and pestle. Samples were then solubilized in a volume of (six times tissue wet weight (WW)) μL lysis buffer containing 9 M urea (Sigma-Aldrich, Stockholm, Sweden), 2 M thiourea (Sigma-Aldrich), 1 mM EDTA (Merck Biosciences, Nottingham, UK), 65 mM DTT (BioRad, Stockholm, Sweden), 0.2 mM PMSF (Amersham Biosciences, Uppsala, Sweden), 0.8 mM benzamidine (Sigma-Aldrich), 25 mM CHAPS (Sigma-Aldrich, 5% Resolyte (pH 4–8) (BDH Laboratory Suppliers, Poole, UK), and 5% Nonidet P40 (Amersham Biosciences). A volume of (0.089 times WW) μL 10% SDS (Shelton Scientific, Shelton, Connecticut), including 33.3% mercaptoethanol (Sigma-Aldrich) and a volume of (0.329 times WW) μL of a solution of DNase I (3.72 U per μL) (Worthington, Lakewood, New Jersey) and RNase A (0.036 U per μL) (Quiagen, Valencia, California), was also added. The mixture was incubated on a shaker at room temperature for 3 h and then centrifuged at 12,000 × *g* to remove any insoluble material. Protein concentration was determined using the Bradford method (BioRad Protein Assay, BioRad) (Bradford, 1976).

Electrophoresis For first dimension, 100 μg total protein were dissolved in rehydration buffer, containing 0.5% (vol/vol) IPG-buffer pH 4–7 (Amersham Biosciences), 7 M urea, 2 M thiourea (Sigma-Aldrich), 65 mM CHAPS, 0.5% Triton X-100 (Sigma-Aldrich), and 18 mM DTT (BioRad) to a final volume of 350 μL and loaded onto an 18 cm IPG-strip pH 4–7 with linear pH gradient (Immobiline DryStrip, Amersham Biosciences). Isoelectric focusing was performed using Ettan IPGphor Isoelectric Focusing System (Amersham Biosciences). Before separation of the second dimension, the gel strips were equilibrated for 15 min with 50 mM Tris-HCl (Shelton Scientific, Shelton, Connecticut), pH 6.8, containing 6 M urea, 30% glycerol, 2% SDS, and 65 mM DTT, to reduce disulfide bonds to allow the proteins to unfold, and 15 min with 50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, and 135 mM iodoacetamide (Sigma-Aldrich) to alkylate thiol groups on proteins. The second dimension was carried out in an 18 × 20 cm SDS polyacrylamide gel with acrylamide:N,N′-ethylenebisacrylamide (37.5:1) (BioRad) with 10%–13% gradient in 192 mM glycine running buffer using Hoefer Dalt 2-D Electrophoresis System gel tank and the Hoefer EPS2A200 power supply (Amersham Biosciences).

Gel staining and image analysis Protein spots were visualized by staining with silver nitrate (Rabilloud *et al*, 1994). A representative gel of guttate psoriasis is shown in Fig 1. Silver stained gels were scanned at 100 μm resolution using a laser densitometer (BioRad), and data were analyzed using the PDQuest software (version 7.1.1, BioRad). Automatic spot detection was performed by PDQuest, which assigned identities according to the Standard Spot number assignment algorithm (SSP). Spot matching was also automatic in the initial phase but complemented with visual landmark assignment (20 spots per gel) and inspection of spot matching in the quality control phase. Final inclusion of spots in the analysis was done after specific quality control criteria were fulfilled. These included; having density measurements above a certain detection threshold, discarding artifacts, checking for local distortions on gels, checking for vector offsets, etc. The inclusion threshold was defined according to spot attributes such as: Gaussian fit, horizontal and vertical gel streaking, degree of spot overlap, and scanner linearity.

Data analysis Different hypotheses concerning the characteristics of the phenotypes were tested by first selecting different subsets of proteins, using PDQuest. Each subset of proteins was formed by selecting proteins with significantly different expression in two specified groups. A protein was considered to have significantly different expression if both Mann–Whitney's test and Student's *t* test yielded a p-value less than or equal to 0.02. The subsets were exported from PDQuest to Excel, where each exported subset consisted of the quantitative values of all spots corresponding to the significant proteins. Quantitative values of absent spots in the exported datasets, which are normally represented as −1, were changed to 1.

The exported datasets were analyzed by hierarchical cluster analysis using the mva and vegan libraries in R (http://cran.r-project.org/). Hierarchical cluster analysis groups similar samples together in an agglomerative way based on a calculated distance matrix (Dysvik and Jonassen, 2001). Here, the distance matrix was calculated using the Bray–Curtis distance function and the clusters were formed using the complete linkage procedure.

Gel staining for protein identification Protein spots with statistically significant variability in the expression pattern between the samples were selected for identification by mass spectrometry. Gels were stained with Coomassie blue or SYPRO Ruby. For Coomassie staining, IPG-strips were loaded with 750 μg total protein and the resulting two-dimensional gels were stained with colloidal Coomassie blue (Sigma-Aldrich). Gels were fixed in 50% ethanol and 2% phosphoric acid (Merck Biosciences, Nottingham, UK) over night and washed 3 times 30 min in deionized water. The gels were then soaked in 34% methanol (Merck Biosciences, Nottingham, UK), 3% phosphoric acid and 1.3 M ammonium sulfate (Sigma-Aldrich) for 1 h before adding Coomassie powder (680 mg per liter). The gels were stained for 4–8 d and were then destained and washed in deionized water. For SYPRO Ruby staining, IPG-strips were loaded with 500 μg total protein and the SYPRO Ruby protein gel stain (BioRad) was used according to the manufacturer's recommendations.

Spot picking and in-gel digestion Protein spots from 2D gels stained with Coomassie blue were picked manually and transferred to 500 μL Eppendorf tubes. The gel pieces were washed overnight in 30% methanol to remove the Coomassie stain. The pieces were then washed 5 min with 50% 50 mM ammonium bicarbonate (Ambic) in 50% acetonitrile (ACN) and briefly washed in ACN before adding trypsin (0.5 μg). Digestion was performed over night at 37°C on a shaker and the peptides were extracted from the gel piece with 50% ACN with 0.1% trifluoroacetic acid (TFA). Samples were completely dried in a vacuum centrifuge and analyzed by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) or liquid chromatography-quadropole time of flight tandem-MS (LC-MS/MS). For MALDI-TOF, samples were dissolved in 2 μL

60% ACN with 0.1% TFA and mixed with 2 μL α-cyano-4-hydroxycinnamic acid dissolved in 50% ACN/50% ethanol (10 mg per mL) and loaded onto a MALDI plate for MALDI-TOF mass spectrometry (Waters, Milford, Massachusetts). Mass spectra were analyzed and matched to SWISS-PROT database entries using Mascot available at http://www.matrixscience.com. Matches were computed using a probability-based Mowse score defined as $-10 \times \text{Log } p$, where p is the probability that the observed match is a random event. Protein scores greater than 72 were considered significant ($p < 0.05$). For LC-MS/MS, samples were analyzed by means of a Waters Micromass modular capillary LC system connected directly to the source of a Q-TOF mass spectrometer (Waters), as described (Allardyce *et al*, 2002). The data were processed by ProteinLynx Global Server v2.0 (Waters) and protein identification was achieved by matching both peptide mass and fragment ion data to proteins contained within the SWISSPROT protein database.

Protein spots from 2D gels stained with SYPRO Ruby were picked automatically using the ProteomeWorks Spot Cutter System with PDQuest Basic Excision Software (BioRad) and placed in 96-well microtiter plates. All spots were then digested using the MassPREP robotic protein handling system (Waters). The protocol for the robotic system is based on in-gel digestion methods as described (Roblick *et al*, 2004). Peptide solutions were concentrated and desalted using a Gyrolab MALDI CD in a Gyrolab Workstation (Gyros AB, Uppsala, Sweden) (Gustafsson *et al*, 2004) and crystallized onto MALDI targets for MALDI-TOF mass specrometry. Mass spectra were analyzed and matched to SWISS-PROT database entries using ProteinProspector available at http://prospector.ucsf.edu/.

We thank Ingrid Eriksson for valuable assistance in performing patch testing. The study was supported by funds from the Swedish Research Council, the Swedish Psoriasis Association, Karolinska Institutet, the Swedish Heart and Lung Foundation and the Welander-Finsen Foundation.

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23501/JID23501sm.htm
Table S1. Identified proteins
Table S2. Patients

DOI: 10.1111/j.0022-202X.2004.23501.x

Manuscript received March 15, 2004; revised July 27, 2004; accepted for publication July 27, 2004

Address correspondence to: Mona Ståhle, Department of Dermatology, Karolinska University Hospital, SE-171 76 Stockholm, Sweden. Email: Mona.Stahle@medks.ki.se

References

Allardyce CS, Dyson PJ, Coffey J, Johnson N: Determination of drug binding sites to proteins by electrospray ionisation mass spectrometry: The interaction of cisplatin with transferrin. Rapid Commun Mass Spectrom 16:933–935, 2002

Anderson L, Seilhamer J: A comparison of selected mRNA and protein abundances in human liver. Electrophoresis 18:533–537, 1997

Asea A, Kraeft SK, Kurt-Jones EA, *et al*: HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442, 2000

Bell S, Degitz K, Quirling M, Jilg N, Page S, Brand K: Involvement of NF-kappaB signalling in skin physiology and disease. Cell Signal 15:1–7, 2003

Bhalerao J, Bowcock AM: The genetics of psoriasis: A complex disorder of the skin and immune system. Hum Mol Genet 7:1537–1545, 1998

Bowcock AM, Shannon W, Du F, *et al*: Insights into psoriasis and other inflammatory diseases from large- scale gene expression studies. Hum Mol Genet 10:1793–1805, 2001

Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254, 1976

Cavani A, Nasorri F, Prezzi C, Sebastiani S, Albanesi C, Girolomoni G: Human CD4+ T lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. J Invest Dermatol 114: 295–302, 2000

Celis JE, Cruger D, Kiil J, *et al*: A two-dimensional gel protein database of non-cultured total normal human epidermal keratinocytes: Identification of proteins strongly up-regulated in psoriatic epidermis. Electrophoresis 11:242–254, 1990a

Celis JE, Cruger D, Kiil J, Lauridsen JB, Ratz G, Basse B, Celis A: Identification of a group of proteins that are strongly up-regulated in total epidermal keratinocytes from psoriatic skin. FEBS Lett 262:159–164, 1990b

Curry JL, Qin JZ, Bonish B, *et al*: Innate immune-related receptors in normal and psoriatic skin. Arch Pathol Lab Med 127:178–186, 2003

de Boer OJ, Verhagen CE, Visser A, Bos JD, Das PK: Cellular interactions and adhesion molecules in psoriatic skin. Acta Derm Venereol Suppl (Stockh) 186:15–18, 1994

Dysvik B, Jonassen I: J-Express: Exploring gene expression data using Java. Bioinformatics 17:369–370, 2001

Eyre RW, Krueger GG: Response to injury of skin involved and uninvolved with psoriasis, and its relation to disease activity: Koebner and 'reverse' Koebner reactions. Br J Dermatol 106:153–159, 1982

Fu H, Subramanian RR, Masters SC: 14-3-3 proteins: Structure, function, and regulation. Annu Rev Pharmacol Toxicol 40:617–647, 2000

Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J: A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 19:359–361, 1998

Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H: Streptococcal throat infections and exacerbation of chronic plaque psoriasis: A prospective study. Br J Dermatol 149:530–534, 2003

Gustafsson M, Hirschberg D, Palmberg C, Jornvall H, Bergman T: Integrated sample preparation and MALDI mass spectrometry on a microfluidic compact disk. Anal Chem 76:345–350, 2004

Hamanaka S, Ujihara M, Numa F, Kato H: Serum level of squamous cell carcinoma antigen as a new indicator of disease activity in patients with psoriasis. Arch Dermatol 133:393–395, 1997

Jonak C, Klosner G, Kokesch C, Fodinger D, Honigsmann H, Trautinger F: Subcorneal colocalization of the small heat shock protein, hsp27, with keratins and proteins of the cornified cell envelope. Br J Dermatol 147: 13–19, 2002

Leffers H, Madsen P, Rasmussen HH, *et al*: Molecular cloning and expression of the transformation sensitive epithelial marker stratifin. A member of a protein family that has been involved in the protein kinase C signalling pathway. J Mol Biol 231:982–998, 1993a

Leffers H, Nielsen MS, Andersen AH, Honore B, Madsen P, Vandekerckhove J, Celis JE: Identification of two human Rho GDP dissociation inhibitor proteins whose overexpression leads to disruption of the actin cytoskeleton. Exp Cell Res 209:165–174, 1993b

Leung-Hagesteijn CY, Milankov K, Michalak M, Wilkins J, Dedhar S: Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin alpha-subunit-binding protein. J Cell Sci 107 (Pt 3):589–600, 1994

Madsen P, Rasmussen HH, Leffers H, *et al*: Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasin" that is highly up-regulated in psoriatic skin. J Invest Dermatol 97:701–712, 1991

Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE: Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. J Invest Dermatol 99:299–305, 1992

Morris SD: Heat shock proteins and the skin. Clin Exp Dermatol 27:220–224, 2002

Nair R, Henseler T, Jenisch S, *et al*: Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. Hum Mol Genet 6:1349–1356, 1997

Naldi L, Peli L, Parazzini F, Carrel CF: Family history of psoriasis, stressful life events, and recent infectious disease are risk factors for a first episode of acute guttate psoriasis: Results of a case–control study. J Am Acad Dermatol 44:433–438, 2001

Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY: Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: A gene microarray analysis. J Allergy Clin Immunol 112:1195–1202, 2003

Oestreicher JL, Walters IB, Kikuchi T, *et al*: Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. Pharmacogenomics J 1:272–287, 2001

Prinz JC: Which T cells cause psoriasis? Clin Exp Dermatol 24:291–295, 1999

Rabilloud T, Vuillard L, Gilly C, Lawrence JJ: Silver-staining of proteins in polyacrylamide gels: A general overview. Cell Mol Biol (Noisy-le-grand) 40:57–75, 1994

Rivas MV, Jarvis ED, Morisaki S, Carbonaro H, Gottlieb AB, Krueger JG: Identification of aberrantly regulated genes in diseased skin using the cDNA differential display technique. J Invest Dermatol 108:188–194, 1997

Roblick UJ, Hirschberg D, Habermann JK, *et al*: Sequential proteome alterations during genesis and progression of colon cancer. Cell Mol Life Sci 61:1246–1255, 2004

Schick C, Kamachi Y, Bartuski AJ, Cataltepe S, Schechter NM, Pemberton PA, Silverman GA: Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. J Biol Chem 272:1849–1855, 1997

Seville RH: Stress and psoriasis: The importance of insight and empathy in prognosis. J Am Acad Dermatol 20:97–100, 1989

Takeda A, Higuchi D, Takahashi T, Ogo M, Baciu P, Goetinck PF, Hibino T: Overexpression of serpin squamous cell carcinoma antigens in psoriatic skin. J Invest Dermatol 118:147–154, 2002

Telfer NR, Chalmers RJ, Whale K, Colman G: The role of streptococcal infection in the initiation of guttate psoriasis. Arch Dermatol 128:39–42, 1992

Thepen T, Langeveld-Wildschut EG, Bihari IC, van Wichen DF, van Reijsen FC, Mudde GC, Bruijnzeel-Koomen CA: Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response *in situ*: An immunocytochemical study. J Allergy Clin Immunol 97:828–837, 1996

Trembath RC, Clough RL, Rosbotham JL, *et al*: Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. Hum Mol Genet 6:813–820, 1997

Turchi L, Chassot AA, Bourget I, *et al*: Cross-talk between RhoGTPases and stress activated kinases for matrix metalloproteinase-9 induction in response to keratinocytes injury. J Invest Dermatol 121:1291–1300, 2003

Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ: The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701–705, 1993

Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L: Psoriasis: A T-cell-mediated autoimmune disease induced by streptococcal superantigens? Immunol Today 16:145–149, 1995

Whyte HJ, Baughman RD: Acute guttate psoriasis and streptococcal infection. Arch Dermatol 89:350–356, 1964

Williams RC, McKenzie AW, Roger JH, Joysey VC: HL-A antigens in patients with guttate psoriasis. Br J Dermatol 95:163–167, 1976

Zhou X, Krueger JG, Kao MC, *et al*: Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. Physiol Genomics 13:69–78, 2003